AlerTox® Sticks

Mustard Seeds

Rapid immunochromatographic test for qualitative detection
of mustard seed antigen in food, kitchens and production facilities.

REF **KIT3040**

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1. Intended Use

The AlerTox® Sticks Mustard Seeds Kit is a rapid, immunochromatographic, lateral flow test for the qualitative detection of mustard seed antigen in food, kitchens and production facilities. Samples that are prepared following the instructions below can be tested using test strips (sticks) from the AlerTox Sticks Almond, Crustacean, Fish, Hazelnut, Mustard Seeds, Sesame and Walnut Kits, but not with other AlerTox Sticks kits. Please read all the instructions before beginning the assay.

2. Introduction

Mustard is a condiment made from the seeds of the mustard plants (white or yellow mustard, *Sinapis alba*; brown or Indian mustard, *Brassica juncea*; or black mustard, *Brassica nigra*). Oriental mustard (also called Chinese mustard, Jie Cai/Gai Choy or *Sisymbrium orientale)* and the wasabi plant (*Eutrema japonicum*) are other members of the mustard family.

Mustard allergies can display a variety of symptoms, ranging from mild oral allergies or hives to severe life-threatening systemic reactions, such as anaphylactic shock or bronchial asthma. Allergy to mustard is one of the major food allergies in some geographical areas, although there is no estimation of overall global prevalence.

The European Food Safety Authority (EFSA) established a list of allergens, including mustard, whose presence in foods must be indicated according to Regulation (EU) No. 1169/2011 Annex II. In the US, the Food Allergen Labeling and Consumer Protection Act (FALCPA) does not include mustard allergies as one of the major food allergies. While some manufacturers include mustard on food labels, it is not required in the US.

3. Test Applications, Specificity and Sensitivity

The AlerTox Sticks Mustard Seeds Kit uses a combination of monoclonal antibodies against a major mustard seed antigen, the legumin-like 11S seed storage protein (cruciferin, CRU4), known as allergen Sin a 2. This kit detects the antigens of all mustard varieties, including wasabi and is suitable for the following applications:

- Food samples
- Rinse water testing
- Surface testing

The limit of detection (LOD) of AlerTox Sticks Mustard Seeds is 2 ppm of raw, unprocessed mustard seed protein (2 mg of mustard seed protein per kg or L of sample).

The range of detection (ROD) for mustard seed protein is 2 – 237,500 ppm of mustard seed protein (mg/kg or mg/L). Overloading (signal decrease) may be seen at 5,000 – 237,500 ppm; however, no total hook effect (false negative) is observed within this range.

View the LODs for surface testing on the Certificate of Analysis (search by lot number at www.hygiena.com/documents).

Section 12 contains the list of matrices currently validated for the kit using an LOD of 2 ppm of mustard seed protein.

AlerTox Sticks Total Mustard is a qualitative assay. To quantify the amount of antigen, use the AlerTox ELISA Mustard Kit (KIT3058).

NOTES:

- AlerTox Sticks Mustard Seeds is highly cross-reactive with seeds of the cruciferous family members, such as broccoli, Brussels sprouts, cabbage, cauliflower, horseradish, radish, rutabaga and turnip.
- The test is non-reactive to rapeseed, canola oil and the edible parts (leaves and roots) of other cruciferous family members.
- The test sensitivity decreases:
    - When the sample has been heated or cooked at temperatures above 150 °C (302 °F)
    - In fat-rich environments (e.g., in the presence of oil or creams)
    - In matrices with low pH (e.g., vinegar or citric acid)
    - When performing the tests at temperatures below 18 °C (64.4 °F)

- Samples that are very viscous, dense or have a high fat content can migrate incorrectly along the chromatography membrane, affecting the assay results (weakening or suppressing test and control lines). Contact us for more information, as these sample extractions may require larger dilutions that affect the LOD (www.hygiena.com/support).

4. Kit Contents

Component	KIT3040
Mustard seed immunochromatographic test strips individually packaged in foil pouches	10
Sample collection tubes with yellow caps	10
Sample extraction buffer in tubes with blue caps, 10 mL	10
Spoons	10
Small pipettes	10
Pipettes, 3 mL (only for testing liquid samples)	10
Swabs (only for testing surfaces)	10

5. Other Materials Not Supplied

- AlerTox Polyphenol Additive (Product No. ASY3213) (only for samples containing polyphenols and antioxidants, such as 70% chocolate*)
- Grinder, mortar or any other manual or automatic homogenization system to crush the sample
- Vortex mixer/shaker (recommended, not required)
- Scissors (only for surface sampling)
- Optional: Digital scale (sensitive to 0.1 g)

* Examples of other foods containing polyphenol and antioxidants include berries, corn fiber, purple corn, legumes (e.g., chickpeas, lentils), soy, tea and wine. These foods need to be validated with the AlerTox Polyphenol Additive.

6. Precautions

- All kit components should be stored at 10 to 30 °C (50 to 86°F).
- Use the test strip within 10 minutes after opening the foil pouch.
- Do **NOT** touch the white end of the test strip.
- Do not use the test strip if it is broken or damaged or if its pouch is torn.
- Do not use the test strips beyond the expiration date.
- All test kit components are disposable; do not reuse them.
- Do not combine components from different kits.

7. Sample Handling

All samples must be at 18 to 35 °C (64.4 to 95 °F) before use.

The test is designed to detect the target antigen in:

- Solid food
- Liquid samples:
  - Beverages
  - Wash water from cutting equipment
- Surfaces

8. Test Procedure for Solid Food Samples

Important: If the sample contains polyphenols or antioxidants (e.g., 70% chocolate, with the exception of coffee), add 1 spoonful of the AlerTox Polyphenol Additive (ASY3213) to a yellow-capped tube and proceed with Step 8.1.

8.1 Before adding the sample to a yellow-capped tube, mash or crush it to obtain the finest crumbs possible. Use a mortar or a grinder, if possible.

8.2 Add 1 g of the sample to the yellow-capped tube.
Alternatively, follow the chart below to add an equivalent amount of sample, using one of the single-use spoons provided.

Food Type	Examples	Spoonfuls
Flours, fine powders	Corn flour, rice flour, milk powder, spices	2
Fine crumbs	Bread, cookies, cakes, snacks	2
Meat, fish and cured meat	Meat, fish, sausage, black pudding, pâté, canned meat and fish	1

8.3 Pour the entire contents of a blue-capped tube (10 mL) into the yellow-capped tube.
Important: Keep the blue cap, as it will be used later.

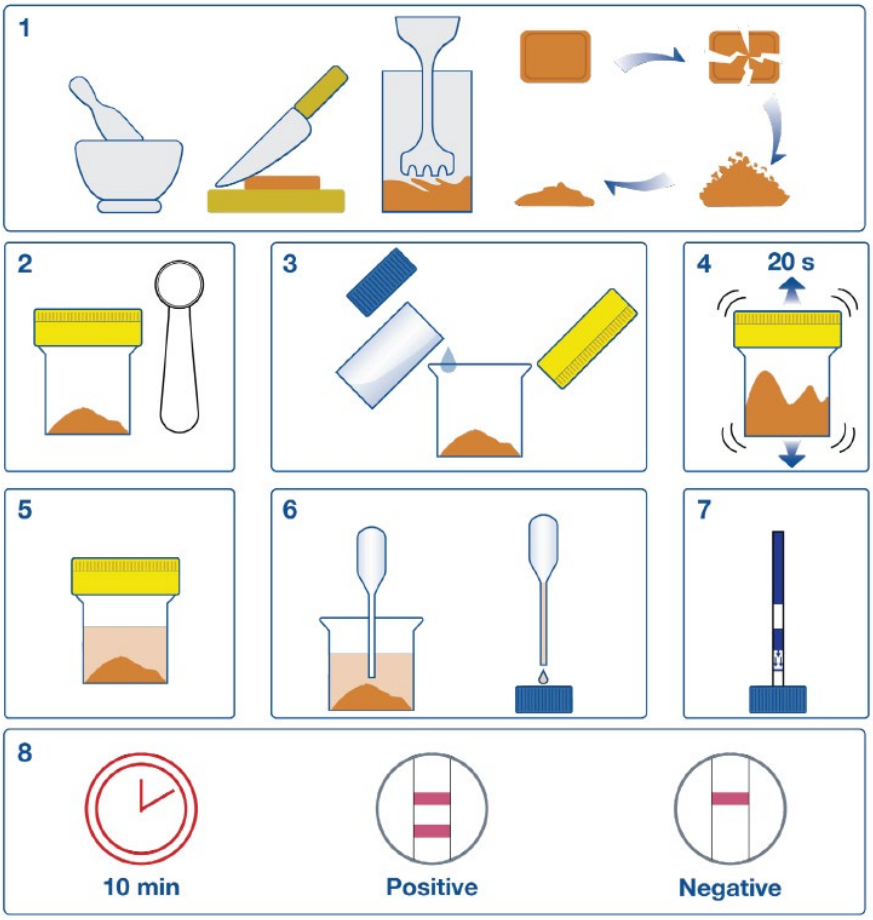
8.4 Close the yellow-capped tube and shake the sample for at least 20 seconds using a vortex mixer to ensure homogenization. Alternatively, shake the tube vigorously by hand.

8.5 Let it rest for 2 minutes so the solids settle.

8.6 Use a small pipette to fill the blue cap with supernatant.
Note: For samples with high fat content, avoid the fat layer of the supernatant.

8.7 Open the foil pouch, carefully remove the test strip by holding its BLUE end and place the white end of the strip in the blue cap.
Note: Do NOT touch the white end of the test strip.

Workflow for Solid Food Samples



8.8 Wait 10 minutes to read the result.
Note: Do not read results after more than 10 minutes, as results may vary. Do not touch the test strip while waiting.

9. Test Procedure for Liquid Samples

Liquid samples – beverages, wash water from kitchen dishes, technological surfaces or cutting machines – may be tested directly. Turbid samples should be filtered (paper or textile filter) or allowed to settle.

Important: If the sample contains polyphenols or antioxidants (e.g., 70% chocolate, with the exception of coffee), add 1 spoonful of the AlerTox Polyphenol Additive (ASY3213) to a yellow-capped tube and proceed with Step 9.1.

9.1 Using a provided 3 mL pipette, add 3 mL of your liquid sample to a yellow-capped tube. If the sample is thick (e.g., yogurt, sauce), follow the chart below to add an equivalent amount of sample to the yellow-capped tube, using one of the single-use spoons provided.

Food Type	Examples	Spoonfuls
Liquid and sauces	Milk, juice, condensed milk, yogurt, soup, gravy, sauce, cream	3

Note: Shake the sample to ensure it is homogeneous and that you are taking a representative test portion.

9.2 Add 3 mL of sample extraction buffer from the blue-capped tube to the sample using the 3 mL pipette.
Important: Keep the blue cap, as it will be used later.

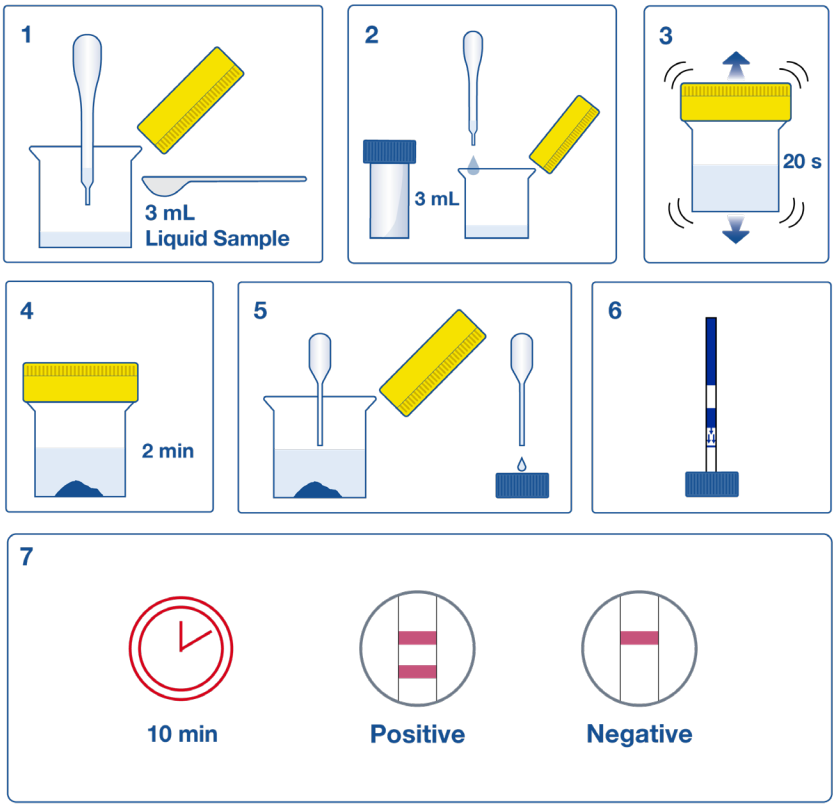
9.3 Close the yellow-capped tube and shake the sample for at least 20 seconds using a vortex mixer to ensure homogenization. Alternatively, shake the tube vigorously by hand.

9.4 If the liquid is cloudy, let it settle for 2 minutes.

9.5 Use a small pipette to fill the blue cap with supernatant.
Note: For samples with high fat content, avoid the fat layer of the supernatant.

9.6 Open the foil pouch, carefully remove the test strip by holding its BLUE end and place the white end of the strip in the blue cap.
Note: Do NOT touch the white end of the test strip.

Workflow for Liquid Samples



9.7 Wait 10 minutes to read the result.
Note: Do not read results after more than 10 minutes, as results may vary. Do not touch the test strip while waiting.

10. Test Procedure for Surface Analysis

Collect each sample using a clean, unused swab. The swab can be used on working surfaces or equipment.

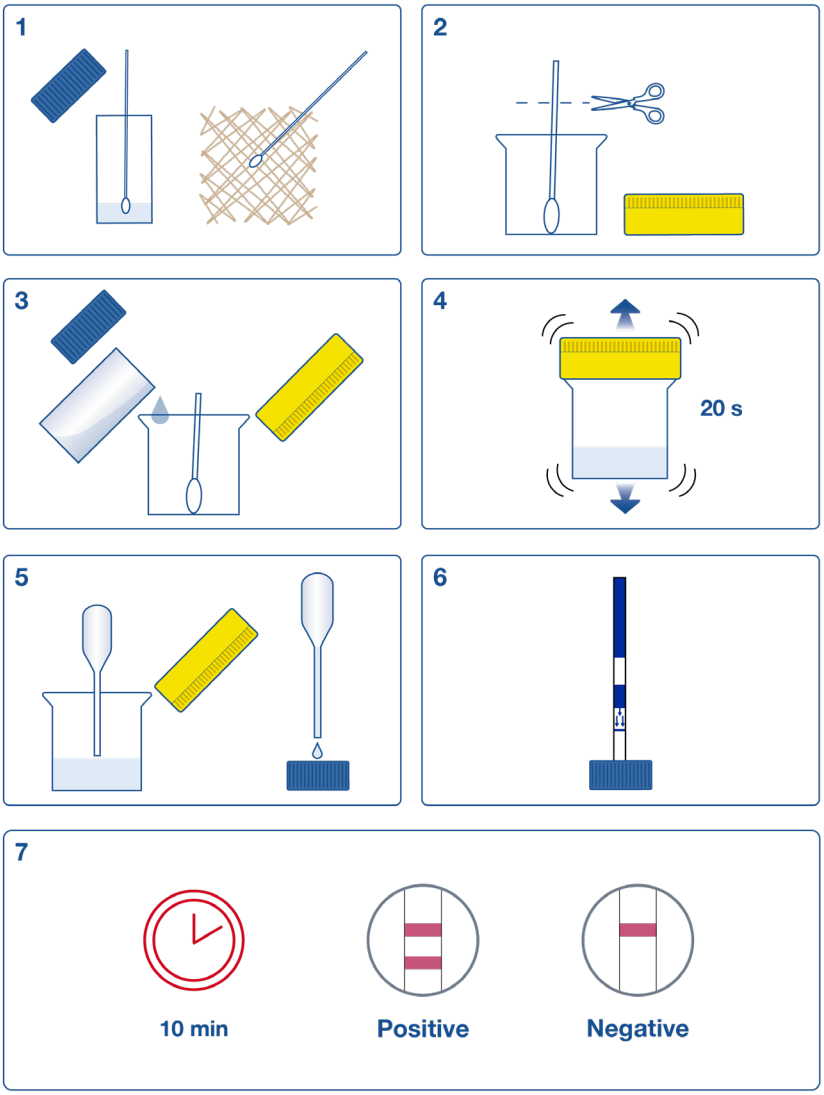
10.1 Wet the swab by dipping it in the blue-capped tube. Then, firmly rub and rotate the swab on the testing surface using a zigzag pattern (at least 16 cm^2/2.5 in^2 or a line of 40 cm/15.6 in).
Note: When possible, swab an approximately 4 cm x 4 cm (1.6 in x 1.6 in) square area. For irregular surfaces, ensure the swabbing technique remains consistent for each test. The area selected for analysis must be representative of the total area of interest.

10.2 Place the swab into the sample collection tube and trim it using scissors.
Note: The swab should fit in the yellow-capped tube when the cap is closed.

10.3 Pour the entire contents of a blue-capped tube (10 mL) into the yellow-capped tube. Then, press the swab tip against the inside wall of the yellow-capped tube to facilitate sample extraction into the buffer.
Important: Keep the blue cap, as it will be used later.

10.4 Close the yellow-capped tube and shake the sample for at least 20 seconds using a vortex mixer to ensure homogenization. Alternatively, shake the tube vigorously by hand.

Workflow for Surface Analysis



10.5 Use a small pipette to fill the blue cap with supernatant.

10.6 Open the foil pouch, carefully remove the test strip by holding its BLUE end and place the white end of the strip in the blue cap.
Note: Do NOT touch the white end of the test strip.

10.7 Wait 10 minutes to read the result.
Note: Do not read results after more than 10 minutes, as results may vary. Do not touch the test strip while waiting.

11. Interpretation of Results

The test result is POSITIVE if TWO colored lines appear: one in the control zone (C) and one in the test zone (T). The color intensity of the test line may vary, but it is not necessarily proportional to the concentration of mustard seed antigen in the sample.



The test result is NEGATIVE if only ONE colored line is clearly visible in the control zone (C).



If NO colored line appears in the control zone (C), the test is INVALID.





If the test is invalid, check for the following and repeat the test with another strip:

- Correct specimen handling
- Correct test procedure
- Expiration date
- Correct storage conditions

For further assistance, contact Hygiena at www.hygiena.com/support.

IMPORTANT NOTE!
AlerTox Sticks is a qualitative test intended to screen samples for internal quality control. Under no circumstances can it replace laboratory analysis testing for quantification.

12. Validation

AlerTox Sticks Mustard Seeds has been validated for the following matrices:

Validated Matrices		
Mustard seeds (black, brown, oriental, yellow)		
Mustard (oriental, ground yellow)*		
Flour (mustard flour, yellow mustard flour, ground brown mustard, cracked yellow mustard seed, cracked brown mustard seed, mixed mustard bran, yellow mustard bran, brown mustard bran)		
Sausage spice mix (salt, vinegar, ground pepper, paprika, cayenne peppers, ground chili, cumin, brown mustard seeds)		
Indian spice mix	Chocolate (70%)†	Coffee‡

* Pure oriental mustard and ground yellow mustard test positive with decreased sensitivity.
† 70% chocolate samples were prepared using the AlerTox Polyphenol Additive.
‡ Although coffee contains polyphenols and antioxidants, coffee samples were NOT prepared using the AlerTox Polyphenol Additive.

Matrices should be validated before use with AlerTox Sticks Mustard Seeds. For additional information about matrix validation, contact Hygiena at www.hygiena.com/support.

13. Disclaimer

Field of use: Use the Hygiena product for research and development, quality assurance and quality control under the supervision of technically qualified persons. The information generated from the Hygiena product is only to be used in conjunction with the user's regular quality assurance program. The Hygiena product should not be used as the sole basis for assessing the safety of products for release to consumers. Data obtained from the Hygiena product must not be used for human diagnostic or human treatment purposes. Before using the product, read the *Limitation of Warranty and Liability* (available in the *Hygiena General Terms and Conditions* at www.hygiena.com/terms-and-conditions).

These products are made from high-quality raw materials. No warranty of any kind is made, either expressed or implied, as to their suitability other than to measure the target antigen content when used exactly in accordance with these instructions, except regarding the quality of these materials.

Use of the kit for any other purpose is outside its intended use. For matrices that have not been previously validated, Hygiena cannot guarantee that the kit is fit for purpose and that the results obtained for these matrices are accurate. Customers may choose to use the product on unvalidated food or surface matrices; however, Hygiena strongly recommends that users perform their own fit-for-use testing to confirm suitability and performance in their specific application. Any damages, including consequential or special damage or expense arising directly or indirectly from using this product, are limited to the replacement value of the kit.

For additional information or assistance with matrix validation, contact Hygiena at www.hygiena.com/support. All Hygiena Terms and Conditions apply and can be found at: www.hygiena.com/terms-and-conditions.

14. Contact Information

For more information, visit www.hygiena.com/contact. For technical support, visit www.hygiena.com/support.

15. Change Index

INS3021 REVB, April 2022
Initial instructions.

INS-KIT3040-001-REVA, July 2025
Updated LOD and ROD information. Included use of the AlerTox Polyphenol Additive for some sample preparations. Standardized branding, wording, some graphic workflows and document ID number.

NS-KIT3040-001-REVB, September 2026
Minor edits

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